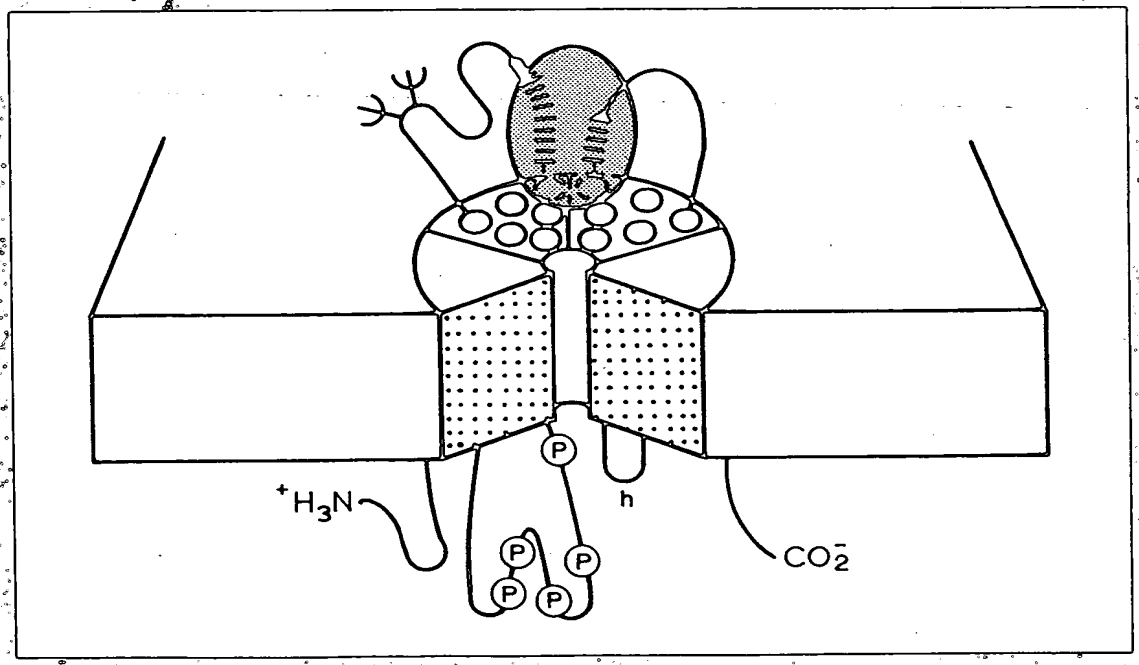
A Textbook of Drug Design and Development

edited by

POVL KROGSGAARD-LARSEN and HANS BUNDGAARD



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PREFACE

Studies of operational and regulatory cell mechanisms are in a state of rapid progess. The introduction of advanced biochemical, biophysical and physicochemical techniques in basic biological research has accelerated the disclosure of the complex mechanisms underlying cell function. Genetic technologies have revolutionized enzyme and receptor research and have made detailed mapping of receptor subtypes possible. Comparative studies of normal and diseased cells *in vivo* and *in vitro* have shed light on the nature of the biochemical and physiological malfunctions characterizing a number of diseases. These studies have disclosed potential targets for therapeutic attack in the relevant diseases.

Natural toxins and analogues of endogenous ligands have been used for the exploration of such sites and their susceptibility to pharmacological manipulation. Lack of specificity does, however, frequently limit the utility of such compounds. Using synthetic and enzymatic techniques it has been possible to convert non-specific toxins or ligands into compounds with highly specific actions on the cellular mechanisms under study. Such specific experimental tools represent the initial steps in the development of therapeutic agents.

Rational and systematic approaches along these lines have provided therapeutically useful drugs and are likely to lead to the development of novel classes of drugs against diseases, which, so far, have escaped effective treatment. In the present textbook all important aspects of modern drug design and development will be described and exemplified.

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1. DRUG DISCOVERY: AN OVERVIEW

MICHAEL WILLIAMS and ALEX M. NADZAN

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13. BIOACTIVE ETHER-LINKED PHOSPHOLIPIDS: PLATELET ACTIVATING FACTOR AND ITS PRECURSORS

FRED SNYDER

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13.1 INTRODUCTION

The ether bond in lipids occurs as either an *O*-alkyl or *O*-alk-1-enyl moiety linked to the *sn*-1 carbon of a glycerol moiety. Usually, the *sn*-2 carbon contains esterified

fatty acids (acyl group) and the *sn*-3 position an esterified fatty acid, phosphate, or phosphobase (P-choline or P-ethanolamine) moiety. However, lyso forms (e.g., diradylglycerols[1], monoradylglycerols, radyllysoglycerophosphocholine, and radyllysoglycerophosphoethanolamine) in addition to other important metabolic intermediates of ether-linked lipids also exist. Glycerolipids containing the *O*-alk-1-enyl-moiety are called plasmalogens. General generic terms that differentiate the 1-alkyl-2-acyl-*sn*-glycero-3-P-, 1-alk-1′-enyl-1-acyl-*sn*-glycero-3-P-, and 1,2-diacyl-*sn*-glycero-3-P- radicals are plasmanyl-, plasmenyl-, and phosphatidyl-, respectively. Therefore, attachment of a base name (e.g., choline or ethanolamine) to the appropriate radical designates a specific subclass of phospholipid, (e.g., plasmanylcholine is 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine). Examples of the different types of common chemical structures that comprise the ether-linked phospholipids with phosphocholine and phosphoethanolamine groupings are illustrated in Figures 13.1 and 13.2. Neutral ether lipids such as 1-alkyl-2,3-diacyl-*sn*-glycerols (the triglyceride analog), 1-alk-1′-enyl-diacyl-*sn*-glycerols (neutral plasmalogens), 1-alkyl-2-acyl-*sn*-glycerols, and 1-alkyl-2-acetyl-*sn*-glycerols are also found in nature or formed as metabolic intermediates in many mammalian cells.

First reports of the occurrence of an ether linkage in glycerolipids appeared in the literature some seventy years ago by Japanese investigators who described the presence of alkyl lipids in several different fish oils. The plasmalogens were also described in the late 1920s by a German group, but their chemical structure was not elucidated until 1957. Although the function of plasmalogens still remains an enigma, their prevalance in most mammalian cells (especially those of the neural and reproductive systems) indicate these lipids are crucial for cellular function. Detailed accounts of the early history of events that led to the identification of the various chemical structures that comprise the ether-linked glycerolipids are provided in some of the reviews and books cited in the reference list.

Perhaps the greatest recent impact on the either lipid field was the discovery of platelet-activating factor (PAF) in 1979 since, before this event, only a handful of investigators were involved in studies of glycerolipids with ether bonds and these were primarily concerned with their biosynthesis and catabolism. It is surprising that such a relative simple chemical structure as PAF, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (Fig. 13.1), could possess such diverse and profound biological activities (see Section 13.5). Moreover, the multitude of cellular responses elicited by this molecule points out the inadequacy of the historically derived term of PAF that describes this new type of phospholipid mediator. As discussed later, it is well established that PAF is a contributing factor in most inflammatory and allergic reactions, but it is also thought to be an important physiological mediator of cellular functions involved in reproduction, fetal development, and blood pressure control. A neutral glycerolipid precursor of PAF (1-alkyl-2-acetyl-*sn*-glycerols) possesses biological properties similar to PAF and these actions are thought to be due to the conversion of alkylacetylglycerols to PAF.

The discovery of PAF resulted in major breakthroughs in the development of anti-PAF drugs by medicinal chemists. Most pharmaceutical companies now have

[1]radyl can represent o-acyl, o-alkyl, or o-alk-l-enyl moieties.

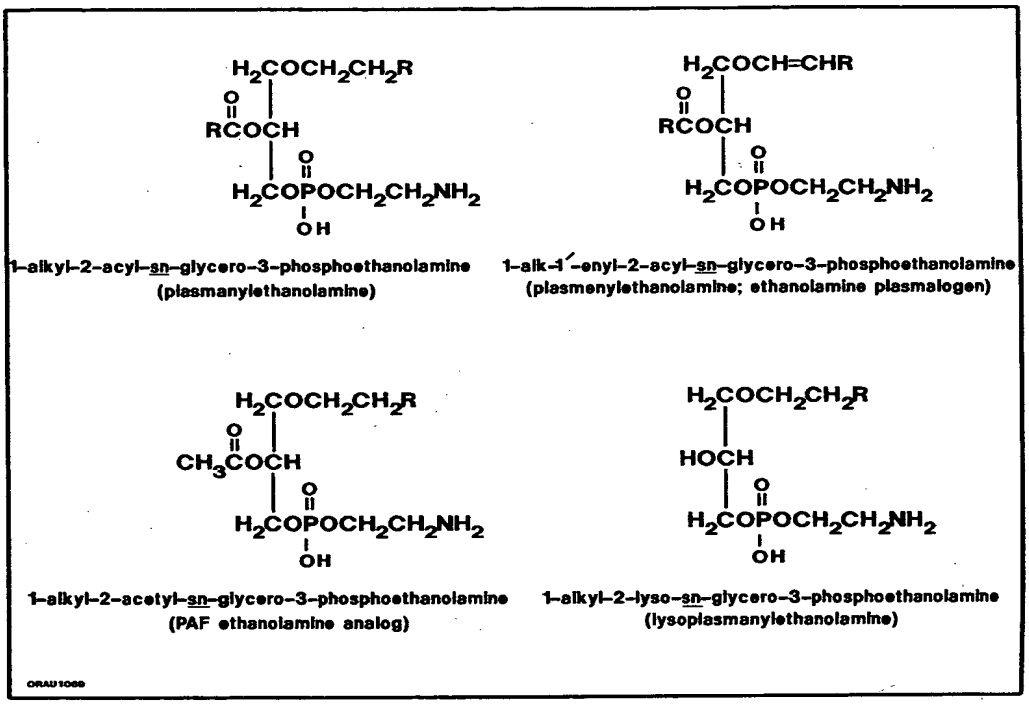


Figure 13.1. Chemical structures of typical ether-linked phospholipids that contain choline.

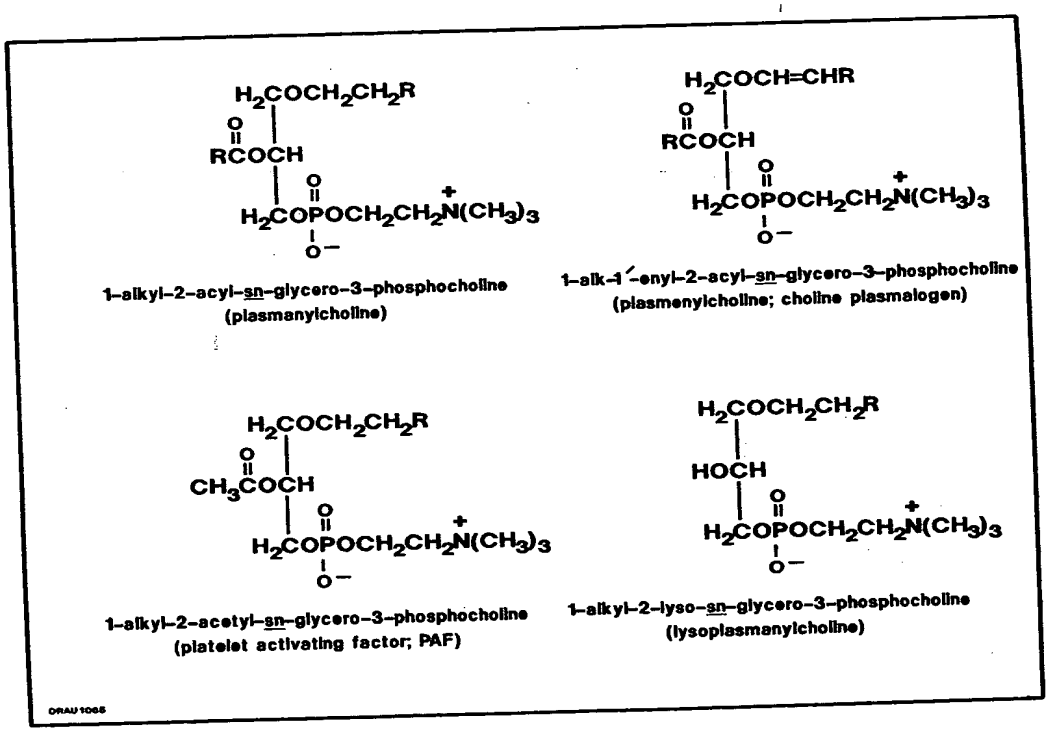


Figure 13.2. Chemical structures of typical ether-linked phospholipids that contain ethanolamine.

an arsenal of PAF receptor antagonists that are being tested in clinical field trials in the treatment of diseases involving inflammatory and hypersensitivity reactions. In fact, receptor targeted antagonists have been synthesized that possess both anti-PAF and anti-histamine activities. Obviously, the magic bullet for treatment of hypersensitivity responses is a drug that has multiple receptor antagonistic properties since the cellular responses leading to inflammation are caused by various types of mediators, including PAF, eicosaniod and phosphatidylinositol metabolites, histamines, etc. Therefore, a future challenge to medicinal chemists interested in inflammatory disease is to successfully design and synthesize new classes of anti-inflammatory antagonists that can interact with more than a single type of agonist receptor.

Potent analogs of PAF have also been described that exhibit preferential hypotensive properties with reduced PAF-like activity towards platelet and neutrophil functions. Thus, unique drugs related to the chemical structure of PAF appear to be on the horizon that could be beneficial in the treatment of hypertension. Similarly, the development of phospholipid analogs that inhibit specific enzymes in PAF biosynthesis would permit greater selectivity for pharmacological intervention of PAF effects than the current generation of receptor antagonists that generally block both the formation of PAF and eicosaniod release from their common precursor. The subject of drug design as it relates to the PAF field is discussed further in the sections of this chapter on structure/function relationships, enzyme inhibitors, and receptor antagonists.

13.2 CHEMICAL REACTIVITY AND CHROMATOGRAPHY

The ether-linkage at the *sn*-1 position imparts a great deal of chemical and metabolic stability to glycerolipids. Nevertheless, both the *O*-alkyl and *O*-alk-1-enyl moieties can be cleaved chemically and enzymatically. The chemical reactivity of the ether linkage will be discussed in this section along with some useful derivatives used in their identification (Figs. 13.3 and 13.4). Those enzymes responsible for the cleavage of ether-linked moieties will be described in Section 13.7 since neither the *O*-alkyl [tetrahydropteridine (Pte · H_4)-dependent alkyl monooxygenase] nor the alk-1-enyl (plasmalogenase) cleavage enzymes have yet been purified for use as analytical tools.

13.2.1 Chemical cleavage of ether-linked moieties

Although both the *O*-alkyl and *O*-alk-1-enyl groups attached to the glycerol moiety of lipids are sensitive to strong acid hydrolysis, the alkyl linkage is much more resistant. The alkyl ether bond can be cleaved by nucleophilic attack of an acid with the formation of an alkyl halide. Hydriodic acid is much more effective in this cleavage than hydrobromic acid or hydrochloric acid. The alkyl iodide product formed in the reaction with hydriodic acid has been used to characterize the ether-linked aliphatic chain by gas-liquid chromatographic analysis. However, other types of derivatives as mentioned later are more advantageous to use because the HI reaction can also form interfering side products (e.g., secondary alkyl iodides produced from olefinic compounds).

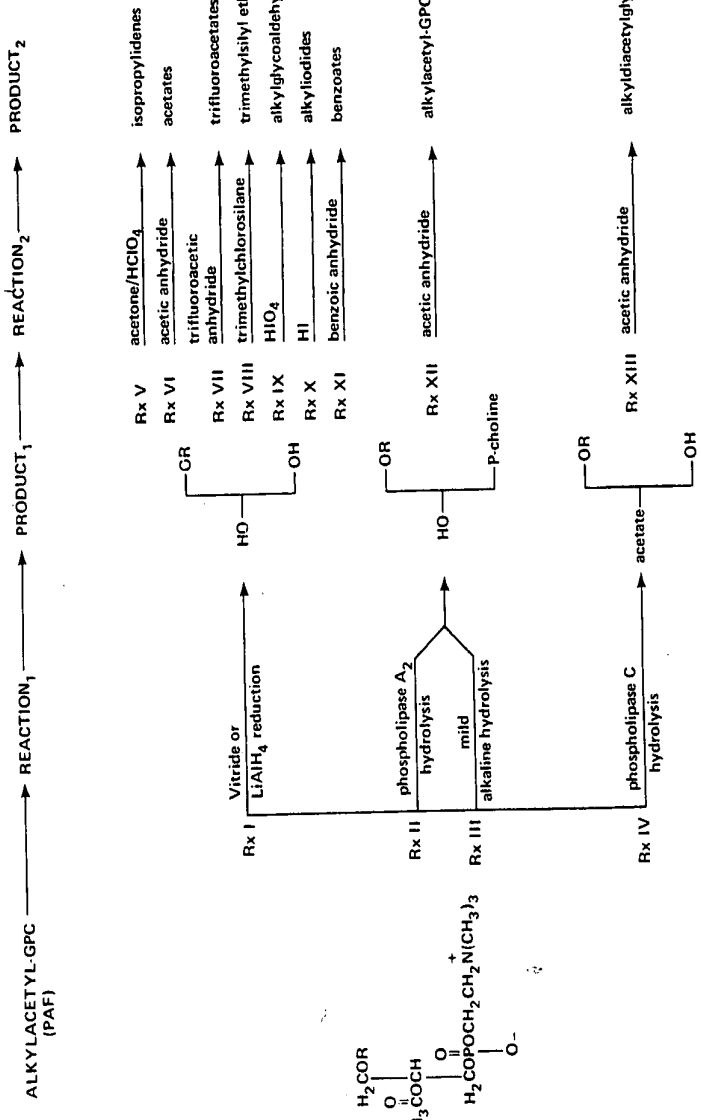


Figure 13.3. Chemical reactions used in the analysis of alkyl forms of naturally occurring ether-linked glycerolipids. The reactions are shown for PAF as the starting lipid; however, the same scheme applies to choline and ethanolamine glycerophosphatides that possess a long chain acyl moiety at the *sn*-2 position.

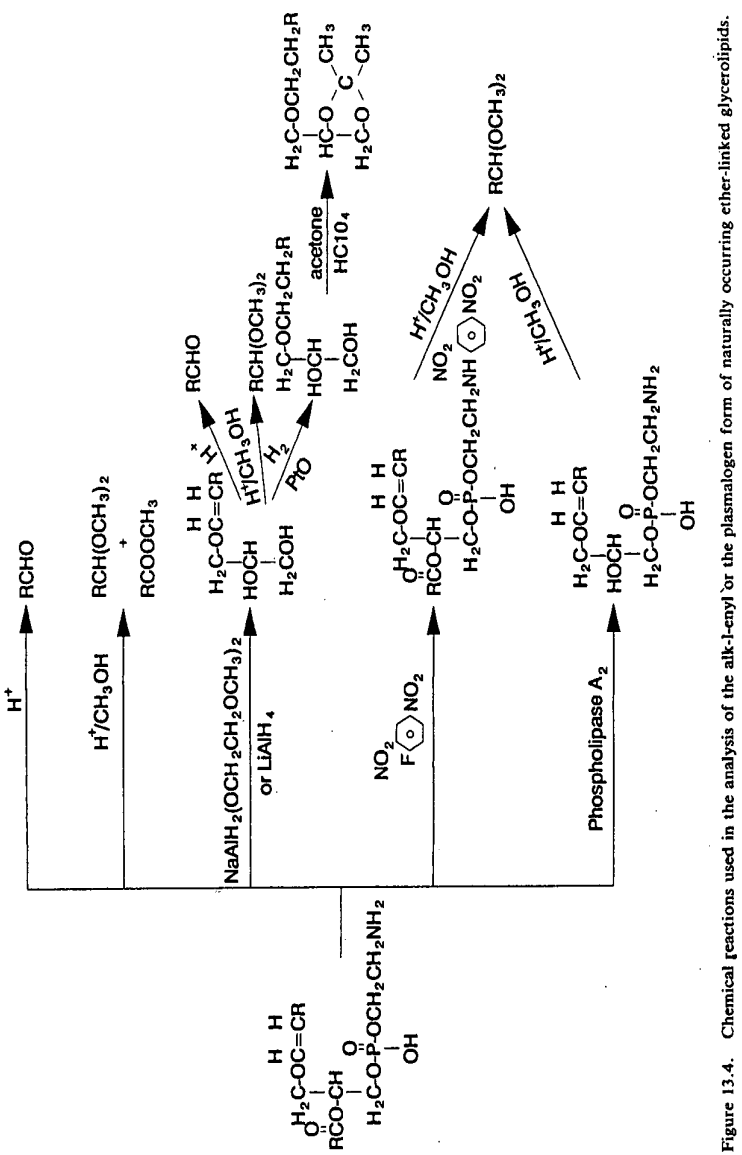


Figure 13.4. Chemical reactions used in the analysis of the alk-1-enyl or the plasmalogen form of naturally occurring ether-linked glycerolipids.

The *O*-alk-1-enyl group in plasmalogens is readily hydrolyzed to fatty aldehydes by any strong acids. If the acid hydrolysis is carried out in the presence of methanol, dimethylacetals are formed, which can be readily characterized by gas-liquid chromatography.

13.2.2 Derivatives of ether-linked glycerolipids at the *sn*-2/*sn*-3 positions and their chromatographic behavior

There are many useful chemical derivatives that can be prepared for identifying specific subclasses or molecular species of ether lipids. The first step always requires the separation of the main classes of lipids in total lipid extracts by adsorption chromatography (thin-layer chromatography or high performance liquid chromatography) before carrying out the appropriate resolution of subclasses and the subsequent chemical reactions to produce useful derivatives for further chromatographic analyses. Neutral lipids can be easily separated into alk-1-enyl-, alkyl-, and acyl-subclasses directly from total lipid extracts, whereas the diacyl, alkylacyl- and alk-1-enylacyl subclasses of phospholipids are not easily resolved by adsorption chromatography. Therefore, with phospholipids it is essential to first remove the phosphobase moiety by using phospholipase C; the products of this enzymatic reaction are diacyl-, alkylacyl-, and alk-1-enylacyl-glycerols. Although these diradylglycerols can be separated from one another by adsorption chromatography, it is generally best to prepare *sn*-3 derivatives of benzoate, acetate, etc., so that migration of the acyl group at the *sn*-2 position is minimized or prevented. Derivatives such as benzoates are extremely useful since they can be easily resolved by chromatography and subsequently quantitated on the basis of their UV absorbing properties.

Another approach for establishing the chain length and degree of unsaturation of ether-linked glycerolipids is to remove the ester groupings substituted at either the *sn*-2 and/or *sn*-3 positions. For example, Vitride and $LiAlH_4$ are excellent reducing agents since they remove all esterified substituents (acyl and phosphobase moieties) without altering the chemical character of the alkyl or the alk-1-enyl ether groupings at the *sn*-1 position of the glycerol moiety. The reduced products, alkylglycerols and alk-1-enylglycerols, can then be characterized as isopropylidene, acetate, or benzoate derivatives by chomatographic analyses.

13.3 PHYSICAL PROPERTIES

Ether-linked phospholipids (alkylacyl- and alk-1-enylacyl subclasses) usually coexist in membranes with their diacyl counterparts. The presence of the ether bond in membrane lipids can influence the biochemical properties of the membrane, especially when the proportion of ether lipids is relatively high. Most studies of the physical properties of ether-linked lipids in membranes have been conducted with artificial model membranes, including monolayer and liposomal preparations. Replacement of ester bonds with ether bonds affect primarily hydrophobic-hydrophilic interactions. However, the closer linear packing arrangements possible with ether-linked chains can also influence the polar head group region of these molecules. The unique location (Δ1) of the double bond adjacent to the ether bond

in plasmalogens also impacts stereochemical relationships and therefore, the two types of ether linkages (alkyl vs. alk-l-enyl) can effect the configuration of membranes in different ways.

Ether lipids in model membranes lead to decreases in ion permeability, surface potential, and lower phase transition temperatures when compared to their diacyl counterparts. However, it is often difficult to generalize the influence of ether linkages of lipids in membranes since the dialkyl analog of phosphatidylcholine has a higher phase transition temperature than the corresponding alkylacyl analog. It is clear that the interpretation of physical effects of ether lipids in biological membranes is extremely complex since the effect of ether bonds on the membrane packing arrangement of cholesterol and proteins can result in secondary alterations in the properties of native membranes.

13.4 NATURAL OCCURRENCE

Ether-linked glycerolipids are found in most mammalian cells and are minor constituents of some plants. Space does not permit a detailed listing of this type of information here; however, reviews by Horrocks and by Sugiura and Waku provide extensive coverage of the topic. These review articles tabulate data compiled from the literature on both the quantity and composition of ether-linked glycerolipids in a wide variety of tissues from numerous species. Table 13.1 illustrates some of the major cells or tissues from various animal species that contain relatively high proportions of alkyl and/or alk-l-enyl types of glycerolipids.

Interestingly, the ether-linked aliphatic chains of tissues have a simple composition in comparison to their acyl counterparts. For the most part usually only saturated or monoenoic aliphatic chains are found in mammals, and 16:0, 18:0, and 18:1 groups predominate. Other types of *O*-alkyl chains have been reported in studies of the molecular species of PAF, but unusual types of alkyl moieties are very minor and their biological significance is questionable. On the other hand it is possible to modify the quantity and chain length/unsaturation composition of both types of ether-linked moieties by feeding appropriate precursors (e.g., fatty alcohols or alkylglycerols) to animals or cells in culture.

13.5 BIOLOGICAL FUNCTION: PHYSIOLOGICAL AND PATHOLOGICAL ASPECTS

Ether-linked glycerolipids serve as structural components of cell membranes and as cellular mediators. The determinant factor in this dualistic function appears to be the chain length of the acyl chain at the *sn*-2 position of phospholipids and the base group at the *sn*-3 position. Those ether-linked phospholipids with long chain *sn*-2 acyl groups (> 12:0) are the structural analogs of phosphatidylcholine and phosphatidylethanolamine and, therefore, they have a comparable role as constituents of cell membranes, albeit differences do exist in their physical and metabolic properties. In contrast, those ether-linked glycerolipids possessing short chain *sn*-2 acyl moieties (< 6:0) exhibit biological activities associated with lipids classified under the generic

Table 13.1 Ether Lipid Subclasses in Choline and Ethanolamine Phosphoglycerides of Mammalian Tissues and Cells

Tissue	Plasmanylcholine	Plasmenylcholine	Plasmanylethanolamine	Plasdenylethanolamine
	percentage of subclass			
Brain	1.0–5.6	tr-4.4	1.1–8.0	50.0–68.1
Erythrocytes	1.9–3.9	0–0.6	2.1–11.5	20.5–59.7
Heart (rat)	0.6–1.4	1.7–3.8	3.2–5.9	12.4–15.1
(species other than rat)	1.6–5.2	33–52	5.8–9.4	42.0–64.1
Kidney	tr-6.2	0–3.7	0–8.8	9.6–33.8
Liver	0–2.4	0–0.14	0–2.0	0–3.7
Lung	2.1–5.9	tr-2.5	4.3–8.7	33.5–50.0
Lymphocytes	10.2–23.2	2.2–3.2	3.9–9.7	40.7–49.9
Macrophages	13.5–35.2	2.3–5.6	0.8–11.4	41.2–61.2
Plasma	0.9–5.5	0–0.2	4.6–20.7	13.8–30.8
Platelets	4.5–18.0	1.4–8.8	1.7–29.1	27.0–57.6
Polymorphonuclear leukocytes	16.4–50.2	tr-9.4	3.0–23.9	32.7–65.5
Spermatozoa	17.1–54.9	23.2–62.8	27.0–49.2	40.0–54.0
Testes	5.6–9.8	0.4–2.6	8.3–16.2	25.5–36.9

Values represent a range for the subclasses of ether-linked phospholipids for tissues and cells from different species and experiments. These data are based on calculations from a detailed review by Sugiura and Waku. Trace quantities are indicated by tr.

term of PAF. Needless to say, the precise physiological functions of ether lipids as membrane components and cell mediators are not fully understood but it is clear that their prominence in most mammalian cells emphasizes their importance.

The diverse nature of the biological properties of PAF can be seen from the list of biological responses induced by PAF (Table 13.2). Stimulation of PAF production via the remodeling pathway of biosynthesis (see Section 13.7) is a contributing factor in inflammatory and allergic reactions and a wide variety of diseases (see following paragraph). On the other hand PAF is also thought to be an important physiological mediator of cellular functions, especially those involved in reproduction, fetal development, and blood pressure control. For example, PAF appears to be required for the successful implantation of the fertilized egg in the uterus. Impressive studies by O'Neill and coworkers in Australia have shown that the treatment of human pre-embryos with PAF causes an increase in the pregnancy rate, which strongly suggests PAF is a controlling factor in pre-embryo development. Furthermore, Johnston and his colleagues (USA) have provided convincing evidence for the requirement of PAF in normal fetal development and parturition. These findings coupled with the implication of PAF as a possible renal factor in blood pressure control indicates that PAF has an essential physiological role, a fact that has often been overlooked because of the considerable emphasis on the inflammatory reactions involving PAF.

PAF has been considered as a contributing factor in a variety of diseases that include asthma, hypertension, acute allergic reactions, anaphylaxis, psoriasis, thrombocytopenic purpura, systemic lupus erythematosus, kidney disorders, pulmonary hypertension and edema, ischemic bowel necrosis, and endotoxin shock. A book edited by Barnes, Page and Henson and a review by Braquet, Touqui, Shen, and Vargaftig are excellent sources of information about the role of PAF in human disease. Perhaps the disease that has received the most emphasis in PAF research is asthma since PAF is the only inflammatory mediator known to sustain bronchial hyper-responsiveness, an important property relevant to asthma. Although there has been some success in clinical trials in treating certain conditions of asthma with PAF antagonists, it appears that more potent and multi-functional anti-PAF drugs must be developed. The production and potent disruptive actions of PAF on renal and

Table 13.2 In Vivo, Tissue, and Cellular Responses or Conditions Induced by PAF

In Vivo	Tissues/Organs	Cellular
Anaphylaxis	↑ Hepatic glycogenolysis	Aggregation (N,P)
Systemic hypotension	Constricts ileum and lung strips	Degranulation (N,P)
Pulmonary hypertension and edema	↑ vascular permeability	Shape changes (P)
↓ dynamic lung compliance		↑ Ca^{2+} uptake (P)
↑ pulmonary resistance		↑ Chemotaxis and chemokinesis (N)
Neutropenia		↑ Respiratory burst and superoxide production (N)
Thrombocytopenia		↑ Protein phosphorylation (P)
Intestinal necrosis		↑ Arachidonate turnover (N,P)
Broncoconstriction		↑ Phosphoinositide turnover (P)

The letters N and P in parenthesis designate neutrophils and platelets, respectively.

gastrointestinal tissues also makes this phospholipid mediator a potentially important underlying factor in the development of diseases involving the kidney and gastrointestinal tract.

13.6 CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY RELATIONSHIPS OF ETHER LIPIDS

Very few modifications can be made in the chemical structure of PAF without a loss in the potency of its biological activity. Replacement of an ester group at the *sn*-1 position greatly diminishes or abolishes bioactivity. Also, the potency of PAF in eliciting cellular responses is proportionately reduced as the *sn*-2 acyl group increases in length beyond three carbon atoms, e.g., PAF analogs with long chain esters at the *sn*-2 position are totally devoid of bioactivity. The same general loss of PAF activity is seen when the choline moiety is altered by the removal of its methyl groups. Dimethyl- and monomethyl-ethanalamine analogs of PAF exhibit moderate PAF activity, whereas 1-alkyl-2-acetyl-*sn*-glycero-3-phosphoethano/amine exhibit no activity. Results obtained with nonhydrolyzable substituents (ethoxy or methylcarbamyl groups) indicate such analogs have PAF-like activity, albeit considerably less than the parent structure; these data suggest the hydrolysis of the *sn*-2 acetate moiety of PAF is not required for biological activity to be expressed.

The possibility that different mechanisms (e.g. different receptor sites) are involved in the hypotensive versus inflammatory properties of PAF has been implicated in studies by Ohno and co-workers in Japan who synthesized a novel PAF agonist, 1(S)-methyl PAF. This small change in chemical structure resulted in an analog that was 2500 times more potent in its hypotensive response than PAF itself; yet it was much weaker in its ability to aggregate platelets or neutrophils. These interesting findings support the notion that beneficial properties of PAF, such as its hypotensive action, might be harnessed through the creation of specific types of PAF analogs.

A number of biologically active ether-linked glycerolipids, other than PAF, also have been reported to occur in biological systems. These include molecules closely related to the structure of PAF, e.g., alkylacetylglycerols, alk-1-enylacylglycerophosphoethanolamines (plasmalogen analog of PAF), acylacetylglycerophosphocholine, and alkylglycerols. Also cyclic acetals and lyso forms of phosphatidic acid analogs have been shown to possess biological activities. Unfortunately, little is known about the structure/function relationships of these lipids and even less about their mode of action.

13.7 METABOLISM

13.7.1 Biosynthesis

PAF and related bioactive cell mediators originate from preformed glycerolipids containing the *O*-alkyl bond. The ether linkage is formed from acyldihydroxyacetone-P and a long chain fatty alcohol through a unique reaction catalyzed by alkydihydroxyacetone-P synthase (Figs. 13.5 and 13.6). No similar type of enzymatic reaction

BIOSYNTHESIS OF ETHER LIPIDS

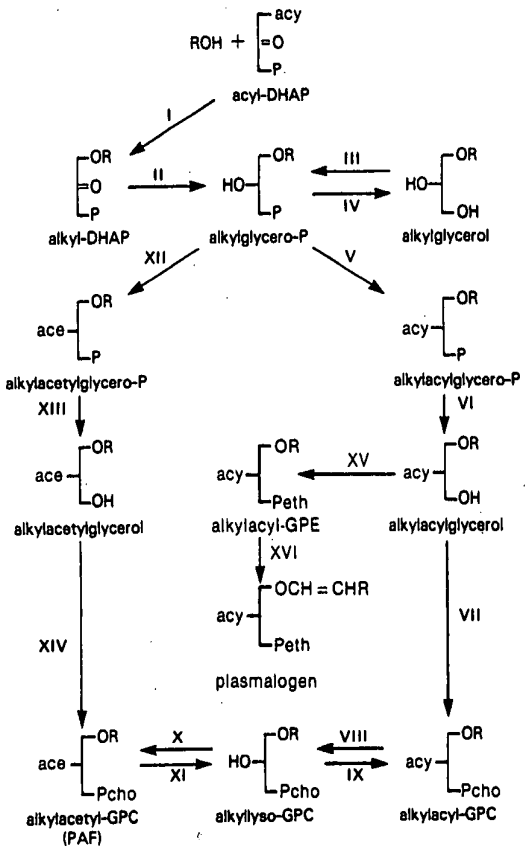


Figure 13.5. Biosynthetic pathways for ether-linked glycerolipids. The roman numerals refer to the following enzymes: (I) alkyl-DHAP synthase; (II) NADPH: alkyl-DHAP oxidoreductase; (III) ATP: l-alkyl-*sn*-glycerol-3-P phosphotranserase; (IV) l-alkyl-2-lyso-*sn*-glycero-3-phosphohydrolase; (V) acyl-CoA: l-alkyl-2-lyso-*sn*-glycero-P-acyltransferase; (VI) l-alkyl-2-acyl-*sn*-glycero-3-P phosphohydrolase; (VII) CDP-choline: l-alkyl-2-acyl-*sn*-glycerol DTT-sensitive cholinephosphotransferase; (VIII) phospholipase A_2; (IX) phosphatidylcholine: l-alkyl-2-lyso-*sn*-glycero-3-phosphocholine polyenoic-specific transacylase (CoA-independent); (X) acetyl-CoA: l-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase; (XI) l-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase; (XII) acetyl-CoA: l-alkyl-2-lyso-*sn*-glycero-3-P acetyltransferase; (XIII) l-alkyl-2-acetyl-*sn*-glycero-3-P phosphohydrolase; and (XIV) CDP-choline: l-alkyl-2-acetyl-*sn*-glycerol DTT-insensitive cholinephosphotransferase; and (XV) alkylacylglycerophosphocholine Δl alkyl desaturase. Abbreviations used are: ace = acetyl; acy = acyl; GPC = *sn*-glycero-3-phosphocholine; and GPE = *sn*-glycero-3-phosphoethanolamine.

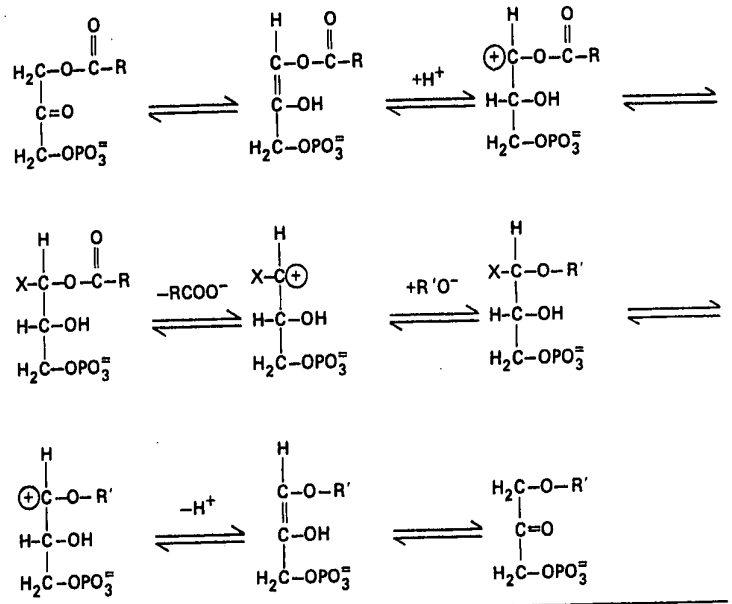


Figure 13.6. Proposed molecular reaction mechanism for the formation of the *O*-alkyl ether linkage by alkyl-dihydroxyacetone-P synthase. X designates the enzyme and the R′ is an alkyl moiety (an H cannot substitute for the R′).

has ever been described in mammalian cells. Studies with a 1000-fold purified alkyldihydroxyacetone-P synthase from Ehrlich ascites cell membranes have suggested that the molecular mechanism of this synthase reaction is of a ping-pong type as illustrated in Figure 13.6. The displacement of the acyl group of acyldihydroxyacetone-P by a long chain fatty alcohol entails the following features (Fig. 13.6): 1) The source of the oxygen that forms the ether bond is the fatty alcohol, 2) The *pro*-R hydrogen at the carbon-1 of the dihydroxyacetone-P moiety exchanges with water with no configurational changes at this position, 3) The acyl group of acyldihydroxyacetone is hydrolyzed before the alcohol substitution occurs, 4) The synthase reaction is reversible as seen by the fact that either fatty acids or fatty alcohols can interact in an exchange reaction with the purported enzyme-dihydroxyacetone-P complex, and 5) A Schiff base intermediate is not formed. Brown and Snyder have suggested a nucleophilic residue of the enzyme (e.g., an amino acid functional group at the active site) might covalently bind the dihydroxyacetone portion of the substrate to form the enzyme-dihydroxyacetone-P complex shown in Figure 13.6.

The fatty alcohol in the reaction catalyzed by alkyldihydroxyacetone-P synthase

is derived from acyl-CoAs by an acyl-CoA reductase and the acyldihydroxyacetone-P is formed via acylation of dihydroxyacetone-P by an acyl-CoA acyltransferase. The acyl-CoA reductase exhibits a high degree of specificity for acyl chains and probably is the factor responsible for the limited types of alkyl and alk-1-enyl chains found in nature.

The initial intermediate formed in the alkyldihydroxyacetone-P synthase reaction (alkyldihydroxyacetone-P) is reduced to the alkyl analog of lyso-phosphatidic acid by an NADPH-dependent oxidoreductase. The product of this reaction, alkyllysoglycero-3-P, is a crucial intermediate that occupies a central branchpoint in the biosynthesis of PAF by the *de novo* and remodeling pathways and in the biosynthesis of those ether-linked lipids found in membranes, e.g., alkylacylglycerophosphocholine and alkylacylglycerophosphoethanolamine, the precursors of PAF and plasmalogens, respectively. The overall reaction steps for the biosynthetic pathway of ether lipids, including the formation of PAF by alternate routes, is illustrated in Figure 13.5.

As seen more clearly in Figure 13.7, PAF can be formed by two separate alternate biosynthetic routes known as the *de novo* and remodeling pathways (PAF cycle of activation/inactivation). *De novo* synthesis of PAF is initiated via the direct acetylation of the alkyl analog of lyso-phosphatidic acid by an acetyltransferase that utilizes acetyl-CoA as the donor. The product of this reaction, 1-alkyl-2-acetyl-1-*sn*-glycero-3-P is then dephosphorylated by a phosphohydrolase to form alkylacetylglycerols; the latter is also a bioactive intermediate and the direct precursor of PAF in the *de novo* route. This final step in the *de novo* sequence involves the transfer of the phosphocholine moiety from CDP-choline to alkylacetylglycerols by a cholinephosphotransferase that is insensitive to dithiothreitol, which is in contrast to the dithiothreitol-sensitive cholinephosphotransferase responsible for the synthesis of phosphatidylcholine. All of the enzymes in the *de novo* pathway of PAF biosynthesis have properties that are distinct from other analogous type enzymes in lipid metabolism. Since the *de novo* route is unaffected by inflammatory stimulatory, it is thought that this pathway maintains PAF levels for essential physiological functions.

The remodeling pathway for PAF production involves an *sn*-2 modification of a membrane phospholipid (alkylacylglycerophosphocholines) via a two-step reaction sequence catalyzed by a phospholipase A_2 and an acetyltransferase (Fig. 13.7). The acetyltransferase must be phosphorylated to be in the active state; however, the precise kinase responsible for this phosphorylation is not firmly established. Enzymes in the remodeling pathway are stimulated by various inflammatory agents and the large quantities of PAF produced under these conditions are thought to be responsible for the severe pathophysiological responses characteristic of all inflammatory diseases.

13.7.2 Catabolism

The ether cleavage enzymes responsible for the hydrolysis of *O*-alkyl and *O*-alk-1-enyl moieties at the *sn*-1 position, PAF acetylhydrolase, and a lyso-phospholipase D that utilizes only ether-linked lyso-phospholipids as substrates are unique to the metabolism of ether lipids. Other esterified substituents attached to the glycerol

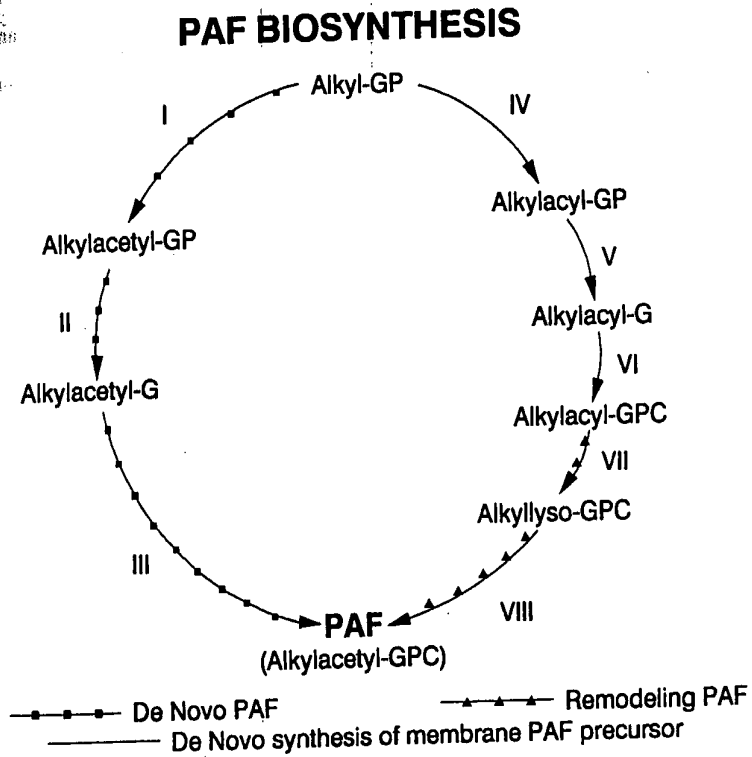


Figure 13.7. Biosynthesis of PAF by dual enzymatic pathways. The roman numerals refer to reaction steps catalyzed by the following enzymes: I. acetyl-CoA: alkyllysoglycero-P acetyltransferase, II. alkylacetylglycero-P phosphohydrolase, III. CDP-choline: alkylacetylglycerol DTT-insensitive cholinephosphotransferase, IV. acyl-CoA: alkyllysoglycero-P acyltransferase, V. alkylacylglycero-P phosphohydrolase, VI. CDP-choline: alkylacylglycerol DTT-sensitive cholinephosphotransferase, VII. Phospholipase A_2, and VIII. acetyl-CoA: alkyllysoglycerophosphocholine acetyltransferase. In this scheme the capital letters correspond to the following groupings: G = glycerol, P = phosphorus, and C = choline.

moiety of ether lipids (e.g., acyl) can be removed by the same enzymes catalyzing the hydrolysis of any other glycerolipid esters, but the ether lipids generally react at slower rates than the corresponding ester analogs. Several recent review articles have covered the pertinent original literature that describes the properties of these catabolic enzymes and their interrelationships with each other. Therefore, only the names of these catabolic enzymes, with a brief synopsis of their function are listed below.

a) **Pte·H_4-dependent alkyl cleavage enzyme.** This Pte·H_4-dependent monooxygenase, which is found primarily in liver, cleaves the *sn*-1 *O*-alkyl linkage in glycerolipids, providing either the *sn*-2 and/or the *sn*-3 of the glycerol portion

position has a free hydroxyl group. The *O*-alkyl moiety is converted to an aldehyde in this reaction.

b) **Plasmalogenase.** This enzyme is responsible for cleaving the *O*-alk-1-enyl grouping in plasmalogens. Plasmalogenase is widely distributed in mammalian tissues, especially in neural cells and the activity appears to be prominent in most cells where plasmalogens exist. Its significance in regulating plasmalogen levels is unknown but the combined action of plasmalogenase and lyso-phospholipase activities could be important in the release of arachidonic acid from plasmalogens. The initial product formed from the *O*-alk-1-enyl moieties in the plasmalogenase-catalyzed reaction is fatty aldehydes.

c) **Phosphohydrolases.** Most phosphohydrolases appear to possess the ability to hydrolyze a free phosphate group from phosphorylated ether-linked glycerolipids. One known exception is the enzyme responsible for the dephosphorylation of 1-alkyl-2-acetyl-*sn*-glycero-3-P since it appears the phosphohydrolase utilizing this lipid as a substrate in the *de novo* pathway of PAF synthesis is highly specific, i.e., phosphatidate phosphohydrolase does not catalyze this hydrolysis. Products of phosphohydrolase reactions can be alkylglycerols, alkylacylglycerols, and alkylacetylglycerols, as well as the corresponding alk-1-enyl analogs.

d) **Phospholipases.** Phosholipases A_2, C, and D all have the ability to hydrolyze esterified substituents attached to ether-linked glycerolipids. Substituents hydrolyzed in these phospholipase reactions are acyl moieties by phospholipase A_2, phosphobase groups (P-choline, P-ethanolamine) by phospholipase C, and base groups such as choline and ethanolamine by phospholipase D.

e) **Lysophospholipases.** Lysophospholipase D is another important enzyme unique to the metabolism of ether-linked glycerolipids. Only alkyllysoglycerophosphocholine and both alkyl- and alk-1-enyl-lysoglycerophosphoethanolamine are known to be substrates for lysophospholipase D. The initial product of the lysophospholipase D reaction is either alkyllysoglycero-P or alk-1-enyllysoglycero-P. However, these phosphorylated intermediates are generally rapidly dephosphorylated to alkylglycerols unless the active phosphohydrolase in such preparations is inhibited by fluoride or vanadate inhibitors.

f) **Lipases.** Pancreatic lipase or similar types of tissue lipases/esterases can catalyze acyl hydrolysis at the *sn*-3 position of 1-alkyl-2,3-diacyl-*sn*-glycerols to produce 1-alkyl-2-acyl-*sn*-glycerols and fatty acids. Studies with pancreatic lipase have focused primarily on its use as an analytical tool and essentially nothing is known about the tissue distribution or physiological significance of neutral lipid lipases that metabolize the alkyldiacylglycerols.

g) **PAF Acetylhydrolase.** PAF is inactivated by a highly specific acetylhydrolase that hydrolyzes the acetate moiety at the *sn*-2 position (Fig. 13.5, Reaction XI). The lyso-PAF produced in this reaction lacks the biological activities of PAF and in most cells the lyso-PAF is rapidly converted to alkylacylglycerophosphocholines that are highly enriched in arachidonic acid and other polyunsaturated acyl moieties. The acylation of lyso-PAF is catalyzed by a Co-A independent transacylase (Fig. 13.7, Reaction IX). PAF acetylhydrolase appears to be distinctly different from a phospholipase A_2 since it is calcium-independent and possesses other unique properties. Both intracellular and extracellular forms of acetylhydrolase exist; although the reactions catalyzed by the two forms are identical, they differ in their characteristics, e.g., the plasma acetylhydrolase has a somewhat higher molecular weight and is resistant to common proteolytic enzymes.

13.7.3 Enzyme inhibitors

Sparse information is available on inhibitors of enzymes involved in the metabolism of ether-linked glycerolipids. Moreover, those described are not highly specific and/or require relatively high concentrations to be effective.

Various isomers of monopalmitoyl-1,2,3-trihydroxyeicosane-1-P have been reported to be inhibitors of alkyldihydroxyacetone-P synthase. Thus, these analogs, at concentrations in the range of 1.3×10^{-4} M, can inhibit the initial step in the formation of an ether-linked intermediate (alkyldihydroxyacetone-P) in the biosynthesis of complex ether-linked lipids. However, with intact cell systems these inhibitors would probably not block ether lipid biosynthesis since the free phosphate moiety of such analogs would make it impossible for them to cross cell membranes.

The other area of ether lipid metabolism where enzyme inhibitors have been described is in the biosynthesis of PAF (see review by Shen and colleagues). Two compounds, developed by these workers at Merck (USA), that inhibit the acetyltransferase activities in the remodeling pathway are 2-[*N*-palmitoyl-amino]propylphosphocholine and 3-[N-palmitoylamino]propylphosphocholine (Fig. 13.8).

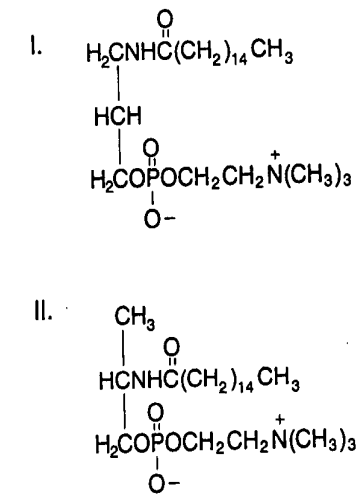


Figure 13.8. Chemical structures of two inhibitors of lyso-PAF acetyltransferase in the remodeling pathway of PAF biosynthesis: I. 2-[*N*-palmitoylamino]propylphosphocholine and II. 3-[*N*[-palmitoyl-amino]propylphosphocholine.

Both inhibit the lyso-PAF acetyltransferase activity in rate spleen microsomes, with IC_{50} values of 5 μM. Interestingly, these synthetic lipids also inhibit the synthesis of PAF by intact mouse peritoneal leukocytes stimulated with calcium ionophore A23187. However, it is likely the inhibition of PAF biosynthesis in intact cells by such amino-containing lipids is also due to inhibition of the phospholipase A_2 that forms lyso-PAF in the remodeling pathway, since it is known that a similar analog, *N*-palmitoylaminoethylphosphocholine, also inhibits acetyltransferase activity as well as phospholipase A_2 activity. Neither of the Merck compounds possess PAF agonistic properties.

Obviously one fruitful area of research in medicinal chemistry would be the development of specific inhibitors for use in mechanistic studies designed to better understand the cellular role of ether-linked lipids. Moreover, highly specific enzyme inhibitors of acetyltransferase and/or phospholipase A_2 in the remodeling pathway of PAF biosynthesis could lead to exciting new drugs (independent of PAF receptors) for the treatment of inflammatory diseases involving PAF. The advantage of enzyne inhibitors over receptor antagonists as drugs to treat PAF-related disease lie in their selectivity. For example, an inhibitor of acetyltranferase in the remodeling pathway of PAF biosynthesis could be used to modulate the level of PAF production via a single reaction step involved in hypersensitivity responses as opposed to the antagonist-induced receptor block that can also interfere with the physiological functions of eicosanoid metabolites.

13.8 MODE OF ACTION OF ETHER LIPIDS AS CELL MEDIATORS: RECEPTORS AND RECEPTOR ANTAGONISTS

Specific PAF receptors have been characterized on the surface membranes of platelets, neutrophils, differentiated HL-60 cells (granulocytic form induced by dimethylsulfoxide), smooth muscle cells, and a cell culture line of murine macrophages ($P388D_1$). Evidence for high affinity PAF binding sites is based on results of [^{3}H]PAF binding and competition experiments with receptor antagonists and structural analogs of PAF. Braquet and Godfroid in France have developed a hypothetical physical model for the PAF receptor binding site (Fig. 13.9). The conformational shape for this site, which accommodates both the lipophilic and hydrophilic portions of the PAF molecule, is based on considerations derived from studies of structural analogs and receptor antagonists of PAF. The number of specific PAF receptors per cell is very low for platelets (estimates of 150–400 per cell in rabbits and 250 in humans) and human neutrophils (1100 per cell), whereas differentiated HL-60 cells possess approximately 5200 specific PAF receptors per cell.

A number of major pharmaceutical firms throughout the world have produced a large number of PAF receptor antagonists by chemical synthesis and through the isolation of compounds from natural products. The variety of PAF antagonists developed by pharmaceutical firms as anti-PAF drugs has been described by T.Y. Shen and co-workers and by Pierre Braquet and collaborators. Examples of the chemical structures of several PAF receptor antagonists are illustrated in Figure 13.10. Antagonists such as CV-3988 closely resemble the chemical structure of PAF,

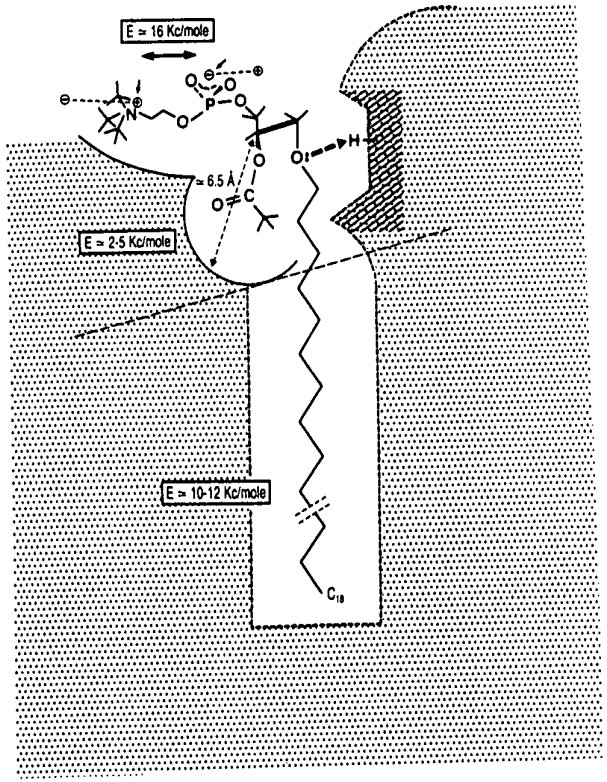
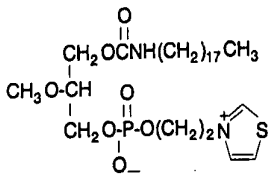


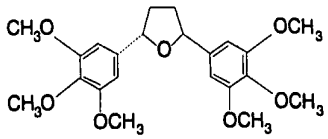
Figure 13.9. Illustration of the putative conformation of the receptor binding site for PAF as envisioned by Braquet and Godfroid. Reproduced from Platelet Activating Factor and Related Lipid Mediators by permission of the authors and Plenum Publishing Corporation, New York.

whereas others such as kadsurenone, L-652, 731, and BN 52021 appear structurally different. Nevertheless, the model of the putative physical constraints for the PAF receptor as proposed by Braquet and Godfroid offers a reasonable explanation of how the receptor might interact with PAF as well as the various receptor antagonists of PAF.

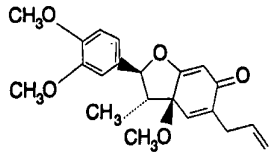
PAF receptor antagonists have been shown to effectively block cellular responses induced by PAF. However, the mechanism of action of PAF on cellular functions via receptor-dependent interactions is still poorly understood. It appears that the interaction of PAF with its receptor affects cellular signal transduction processes involving GTP regulatory proteins and possibly the adenylate cyclase system, since PAF is known to stimulate GTPase activity and to inhibit adenylate cyclase. Also, the significant qualities of PAF retained by some cells imply that PAF might function as an intracellular mediator. Clearly much more research is required to sort out the



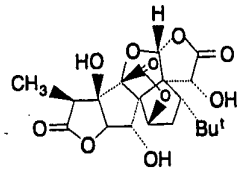
CV-3988



L-652,731



Kadsurenone



Ginkgolide B
(BN 52021)

Figure 13.10. Chemical structures of several commercial PAF receptor antagonists.

hierarchial roles and the exact biochemical mechanism(s) involved in the complex interrelationships of PAF and the various bioactive metabolites of phosphoinositides and arachidonic acid.

The design of antagonists or enzyme inhibitors as drugs for multiforms of biologically active glycerolipids related to the PAF structure are areas of future potentially lucrative research in the field of medicinal chemistry. As mentioned earlier, the *sn*-1 alk-1-enyl (plasmologen) and *sn*-1 acyl analogs of PAF, alkylacetylglycerols, alkylglycerols, and cyclic acetals and lyso forms of phosphatidic acid analogs can also induce cell-mediated responses and all of these agonists are known to be produced by certain mammalian cells. Although the enzymes that form and degrade most of these PAF related mediators have been described, very little information is available about the mechanisms responsible for their biological activities. The complexity of the possible interactions of the various glycerolipid mediators will only be forthcoming if highly specific enzyme inhibitors can be developed as tools to delineate the structural-functional relationships of each type of glycerolipid agonist. The *modus operandi* for such investigations are analogous to the principles used earlier in sorting out the properties and biological importance of the variety of eicosanoid metabolites produced by different cell types.

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